**Name of journal: World Journal of Methodology**

**ESPS Manuscript NO: 7444**

**Columns:** **Editorial**

**Prospects and advancements in C-reactive protein detection**

Chandra P *et al.* CRP detection technologies

Pranjal Chandra,Pankaj Suman, Himangi Airon, Monalisa Mukherjee, Prabhanshu Kumar

**Pranjal Chandra,Pankaj Suman, Himangi Airon, Monalisa Mukherjee, Prabhanshu Kumar,** Biomimetic Research Laboratory, Amity Institute of Biotechnology, Amity University Uttar Pradesh, Noida 201303, India

**Author contributions:** All authors made a substantial contribution to the conception and design of manuscript, drafting the article or revising it.

**Correspondence to: Pranjal Chandra, Assistant Professor,** Biomimetic Research Laboratory, Amity Institute of Biotechnology, Amity University Uttar Pradesh, J-3 BlockSector-125, Gautam Buddha Nagar, Noida 201303, India. pchandra1@amity.edu

**Telephone**: +91-120-4392644 **Fax**: +91-120-4392295

**Received:** November 19, 2013 **Revised:** January 13, 2014

**Accepted: February 16, 2014**

**Published online:**

**Abstract**

C-reactive protein (CRP) is one of the earliest proteins that appear in the blood circulation in most of the systemic inflammatory conditions and this is the reason for its significance even after identification of many organ specific inflammatory markers, which appear relatively late during the course of disease. Earlier methods of CRP detection were based on the classical methods of antigen-antibody interaction through precipitation and agglutination reactions. Later on CRP based enzymatic assays came into the picture which got further modified by integration of antigen-antibody detection system with surface plasma spectroscopy. Then came the time for the development of electrochemical biosensors where people used nano-materials to make highly sensitive and portable detection system based on silicon nanowire, metal-oxide-semiconductor field-effect transistor/bipolar junction transistor (MOFSET/BJT), ZnS nanoparticle, aptamer, field emission transmitter, vertical flow immunoassay *etc*. This editorial attempts to summarize development in the field of CRP detection with a special emphasis on biosensor technology. This would help in translating the latest development in CRP detection in clinical diagnosis of inflammatory conditions at an early onset of the diseases.

© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

**Key words:** C-reactive protein; Inflammation; Diagnostic methods; Antibody; Biosensors

**Core tip:** Over the time C-reactive protein has emerged as one of versatile marker for the detection of systemic inflammatory conditions and providing preliminary information to the clinicians to go for more specific diagnostic methodology to follow. Advancements in the electroanalytical chemistry and knowledge of nanomaterials have helped the modern age researchers to miniaturize detection system with enhanced level of specificity and sensitivity of C-reactive protein (CRP) detection. Further research should be directed in this area to devise better diagnostic platform that can detect the change in CRP level at a very early level of the onset of inflammatory conditions.

Chandra P,Suman P, Airon H, Mukherjee M, Kumar P.Prospects and advancements in C-reactive protein detection.

**Available from:**

**DOI:**

**INTRODUCTION**

In humans, there are many acute phase proteins whose level in blood plasma increases or decreases in response to inflammation (acute phase reaction). Some of the acute phase proteins are C-reactive protein, mannose binding protein, complement factors, serum amyloid A, fibrinogen, retinal binding protein, ceruloplasmin, and antithrombin. Amongst them, CRP is the most important, sensitive and systemic marker of inflammation identified in the human body as its level rises rapidly in the blood plasma in response to large number of foreign bodies, infections, tissue damage, renal disease as well as cardiovascular diseases[1]. It is secreted by hepatocytes in response to cytokines like interlukin 6, interlukin 1, tumor necrosis factor alpha *etc*[2]. CRP (Mr 115,135), a member of pentraxin family of calcium dependent ligand binding plasma protein, is composed of 5 non-glycosylated polypeptide subunits, each of which is composed of 206 amino acid residues. Polypeptide units associate to each other through non-covalent bonding in an annular configuration forming cyclic pentameric symmetry. The ligand binding site of CRP comprises of loops with two calcium ions. During inflammation, phosphocholine present on necrotic or apoptotic cells bind at the active site of CRP, thereby activating the classical complement pathway essential for opsonisation and induction of pro-inflammatory patho-physiological effects. Additionally, it also activates complement pathway but, also increases respiratory burst of neutrophils, encourages expression of adhesion molecules and synthesis of tissue factors. Based on these clinical importances of CRP, attempts have been made in this editorial to summarize the chronological development in the field of CRP detection. Physiological level of CRP in the human plasma is 2mg/L whereas, during inflammatory conditions, its concentration raises significantly in 6-8 h that can even reach up to 300 mg/L in next 48 hours. CRP level in patients with cardiovascular disorder, and/or myocardial infarction at the time of admission to the hospital have been observed to be above the physiological range (more than 3mg/L)[3]. CRP deposits in the arterial walls during atherogenesis thereby activating complement pathway, and augmenting the development of several cardiovascular disorders[4]. Abraham *et al*[5] observed a higher level of CRP (14.3 mg/L +/- 11.2 mg/L) in the patients before dialysis who were susceptible to chronic kidney disorder, renal failure or kidney malfunction. Higher concentration of CRP is also found during late pregnancy. People with obesity and high BMI (body mass index) also have higher level of CRP in the blood plasma[6]. In a study by Seounghee *et al*[7] raised level of high sensitivity CRP (hsCRP) was also correlated with the development of cancer. Hence, CRP is an important marker of clinical conditions like local and systemic inflammation, myocardial diseases, obesity *etc*. A prospect to develop a highly specific and sensitive method of detection of CRP at an early stage of these clinical conditions has been attempted by various research groups. The overall chronological development has been elucidated in the figure 1.

Conventional methods of CRP detection rely on precipitation by C-polysachharide of *Pneumonococcus*, tube precipitation, complement fixation, latex agglutination, radioimmuno assay (RIA), radial immunodiffusion, and fluorescence polarization. Detection of CRP by radial immunodiffusion uses radial immunodiffusion plates made of agarose containing 1% rabbit anti-human CRP in it. Sera samples were added in the wells punched on them and the diameter of the radial rings were measured after 48 h incubation period. Greater the diameter of the precipitation ring more is the CRP concentration in the serum. Time taken for the assay and it’s semi-quantitative nature are the major limitations of this detection system[8]. As an improvement of the previous technique, latex agglutination method has been developed which employs inert latex particles coated with anti-human CRP antibody. In presence of CRP in the patient’s serum, agglutination reaction can be noticed between anti-human CRP and CRP moieties. Unlike the precipitation reaction it takes less time but still holds the limitation of being semi-quantitative in nature[9]. In 1990, Kurosawa *et al*[10] developed a latex piezoelectric immunoassay using piezoelectric quartz crystal which acts as the sensing element for the change in viscosity or density in the solution due to aggregation of latex particles. It negated out the disadvantages of previous methods of detection of CRP using agglutination, through the use of latex bearing antibody without any film. Earlier piezoelectric assays employed the formation of antibody coated thin film latex on a crystal by which the oscillating frequency of the crystal reduces. This approach removed the drawbacks of previous methods in terms of labeling reporter molecule and also through improving the assay sensitivity. Further, immuno-enzymometric assay for determination of CRP using two antibodies has been developed by Käpyaho *et al*[11] It is a simple assay consisting of single immunological reaction between CRP, and peroxidase labelled antibody with another antibody attached to the wall of test tube. The immune complex formed is determined by a colorimetric assay using peroxidase substrate. Sensitivity of this technique was comparable to the turbidimetric method of CRP detection. However, concerns about enzyme stability, shelf life, and time taken for detection raises the question about its practical applications and shelf life of the diagnostic system[11]. An ELISA kit for the detection of CRP (Cell Biolabs Inc., San Diego, CA, United States) has anti-CRP antibody coated onto the microtitre plate that reacts with the CRP antigens. Enzyme linked secondary antibody in the presence of specific substrate gives rise to a colorimetric reaction whose optical density can be measured to estimate the level of CRP. The detection limit of this was up to 0.1 ng/mL, but high false positives due to non-specific binding limits the availability of this methodology. Another major disadvantage include the long detection time, less sensitivity, low stability, cross reactivity with the serum proteins, lack of miniaturization, and on-site analysis.

Thus, in recent years, various biosensor based detection system have been attempted for quick, sensitive, and on-site detection of CRP. Biosensor is an analytical device utilizing a biological reaction between receptor and target molecules, converting the biological response into readable and quantifiable signals using transducers[12-14]. Kim *et al*[16] developed a biosensor based on surface plasma resonance spectroscopy which involved measurement of molecular interactions at the gold/silver surface of the sensing element thereby measuring reflectance of light with respect to the refractive index of the surface of biosensing element that changes when CRP molecular species react at the fabricated unit. This technique uses poly(3-(2-((N-succinimidyl)succinyloxy)ethyl)thiophene)(P3SET) which is a polythiophene with pendant N-hydroxysucciniamide (NHS) easter group as a biolinker between anti-CRP (bioreceptor) and sensing surface. Self-assembled monolayer (SAM) of P3SET was formed on the gold surface and anti-CRP was immobilized covalently. When CRP reacted with sensor, there was a shift in the refractive index of P3SET/anti-CRP due to formation of P3SET/anti-CRP/CRP on the sensing surface, and reflectance deviated. Hence, reaction between anti-CRP immobilized on gold surface, and CRP can be monitored using surface plasma resonance with high sensitivity[15].

With advancements in nanotechnology, nanobio-sensors are becoming very popular in recent times. In this regard, Lee *et al*[7] attempted the Silicon-Nanowire based fabrication process which follows a top-down approach of fabrication using micro-machining technology. In a new study, Yuan *et al*[17] developed a method to adjust sensitivity using a gated lateral bipolar junction transistor (BJT) in the metal–oxide–semiconductor field-effect transistor-bipolar junction transistor (MOSFET–BJT) hybrid mode which was fabricated using complementary metal–oxide–semiconductor (CMOS) manufacturing system. Si3N4 was immobilized on the layer on gold which were then immobilized on floating gate using electron beam evaporator. A die chip consisting of gated lateral BJT was then embedded onto a printed circuit board (PCB) which was further connected to vertical collector, base and lateral collector, and emitter. Internal metal layers were also employed to enhance rate of current flow. Monoclonal anti-CRP antibodies were linked to gold layer using self-assembled monolayers of 11-mercaptoundecanoic acid, N-Hydroxysuccinimide, and N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride. On reaction with CRP species, capacitance between the liquid and floating gate changes that get measured. This change in capacitance has been used to determine the concentration of CRP with high sensitivity and reliability. The advantages of such a system were small size, ease of manufacturing, low noise, high transconductivity, good selectivity, and reproducibility. It has also been claimed that the developed system can also be used for other biomarkers too by only changing the corresponding antibody.

Biosensor integrated with microfluidic device has been also developed for the detection of CRP. In a report, CRP along with other cardiac marker c-troponin has been detected simultaneously using a microfluidic device. The device developed chip acted as a micro-reactor for the simultaneous detection of CRP and c-troponin. Antibodies with bio-conjugated CdTe and ZnSe were used in the system. These quantum dots release Zn2+ and Cd2+ ions that get detected by square-wave anodic stripping voltammetry to enable the quantification of the two biomarkers. This electrochemical immunosensor has a detection range of 0.5-200 µg/mL with detection limit of 307AMOL in 30 µL for CRP[18]. Another method of detection which uses Zn2+ ions for the detection of CRP was established by Chad *et al*[19] where ZnS nanoparticles were used to transduce the signal *via* fluorescence spectroscopy. In this detection system, mouse anti-CRP coated magnetic microbeads were used. On addition of the serum sample containing CRP, immune complex bind to these beads to which biotinylated mouse anti-CRP will fix. To this complex, neutravidin conjugated with ZnS nanoparticles will attach which in the presence of Flouzin3, a zinc ion selective fluorescence dye, generate fluorescence signal. The bioassay possesses a detection limit of 10 pmol which makes it highly sensitive method to detect CRP. In addition, it is also non-toxic and less expensive system to fabricate. Another biosensor based on nanomaterial for the detection of CRP level was developed by Qureshi *et al*[20] The detection system required the use of specific interaction between CRP and its correspondence RNA aptamer. These CRP specific RNA aptamers were immobilized on carbon-nanotubes (CNT) activated gold interdigitated electrodes of capacitors *via* a physical adsorption. The selective binding of RNA aptamers with CRP was determined by measuring the capacitance after competitive binding between complementary RNA (cRNA) and CRP in pure forms, and co-mixtures. It is a label-free method of detection based on affinity separation of target molecules with limit of detection ranging from 1-8 µM. Although the detection limit was very low, this method has merit in terms of a label-free approach and simple approach for detection of CRP. Chang-hoon *et al*[21] recently developed a biosensor using field effect transistor (FET) in which silicon binding protein (SBP) were linked to surface protein A to simplify the tedious method of fabrication of monolayer. SBP, an artificial protein, can bind to silicon surface without any bi-linker. Fabricated device was treated with hot piranha solution to maximize the affinity of SBP-protein A complex onto the sensing area. The SBP-protein A is then immobilized on the surface of sensing element and dipped into the solution containing anti-CRP. The anti-CRP gets coated onto the fabrication unit where CRP forms the immune complex which is transduced in a detectable signal. This is the application of a biosensor point-of-care-testing system with detection limit comparable to that of ELISA. Oh *et al*[22] lately developed a one-step biosensor for high sensitivity CRP (hsCRP) detection using vertical flow immunoassay (VFA). It is composed of a sample pad, FTH (flow thorough films) film, conjugate pad, and nitrocellulose membranes (onto which anti-hsCRP and secondary antibodies are immobilized below the holes) are stacked upon one another. Anti-hsCRP conjugated with gold nano-particles was encapsulated in the conjugate pad. This fabricated system detects hsCRP 0.01-10 µg/mL within 2 min, and is the most rapid biosensor till date.

Recently, an optimized biosensor for a label-free detection of CRP in blood serum sample has been developed by Bryan *et al*[23] which are based on electrochemical impedance spectroscopy (EIS) using gold electrodes. SAMs of polyethylene glycol (HS-C11-(EG)3-OCH2-COOH) with the help of ethanol and nitrogen gas were made and dipped into piranha solution. NHS was used to activate carboxylate group and monoclonal anti-CRP was linked to monolayers covalently. This device detects CRP in blood on the basis of difference in impedance when CRP species that reacts with monoclonal anti-CRP antibody bound to SAM. This system of detection has very good selectivity and reusability with no loss of apparent sensitivity. This can be considered as one of the latest methods of CRP detection where no specific labeling is required *i.e.* a label free detection system even though pico molar detection limit.

**CONCLUSION**

Our understanding of C-reactive protein detection systems has come a long way. Over the years CRP has become a versatile inflammatory marker for the detection of systemic inflammatory conditions. In future, advancements in the interdisciplinary approaches such as will be helpful for the quick, ultrasensitive analysis of these markers. Attempts should also be made for developing new CRP recognition molecules and new material to develop the sensing platforms. While development and implementation these concepts, care should be taken that these systems should have promise for CRP analysis in body fluids.

**ACKNOWLEDGEMENT**

Authors thanks Amity University Uttar Pradesh, Noida, India for providing the research facility.

**REFERENCES**

1 **Pepys MB**, Hirschfield G M. (2003 June). C-reactive protein: a critical update. *J Clin investiga 2*003; **2**: 1805-12 [DOI: 10.1172/JCI200318921]

2 **Thompson D**, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure* 1999; **7**: 169-177 [PMID: 10368284 DOI: 10.1016/S0969-2126(99)80023-9]

3 **Li JJ**, Fang CH. C-reactive protein is not only an inflammatory marker but also a direct cause of cardiovascular diseases. *Med Hypotheses* 2004; **62**: 499-506 [PMID: 15050096 DOI: 10.1016/j.mehy.200312014]

4 **Prasad K**. C-Reactive Protein and Cardiovascular Diseases. *Int J Angiol* 2003; **12**: 1-12 [DOI: 10.1007/s00547-003-1018-y]

5 **Abraham G**, Sundaram V, Sundaram V, Mathew M, Leslie N, Sathiah V. C-Reactive protein, a valuable predictive marker in chronic kidney disease. *Saudi J Kidney Dis Transpl* 2009; **20**: 811-815 [PMID: 19736479]

6 **Visser M**, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. *JAMA* 1999; **282**: 2131-2135 [PMID: 10591334 DOI: 10.1001/jama.282.22.2131]

7 **Lee S**, Choe JW, Kim HK, Sung J. High-sensitivity C-reactive protein and cancer. *J Epidemiol* 2011; **21**: 161-168 [PMID: 21368452 DOI: 10.2188/jea.JE20100128]

8 **Harris RI**, Stone PC, Hudson AG, Stuart J. C reactive protein rapid assay techniques for monitoring resolution of infection in immunosuppressed patients. *J Clin Pathol* 1984; **37**: 821-825 [PMID: 6430971 DOI: 10.1136/jcp.37.7.821]

9 **Senju O**, Takagi Y, Uzawa R, Iwasaki Y, Suzuki T, Gomi K, Ishii T. A new immuno quantitative method by latex agglutination--application for the determination of serum C-reactive protein (CRP) and its clinical significance. *J Clin Lab Immunol* 1986; **19**: 99-103 [PMID: 3514917]

10 **Kurosawa S**, Tawara E, Kamo N, Ohta F, Hosokawa T. Latex piezoelectric immunoassay: detection of agglutination of antibody-bearing latex using a piezoelectric quartz crystal. *Chem Pharm Bull (Tokyo)* 1990; **38**: 1117-1120 [PMID: 2393945 DOI: 10.1248/cpb.38.1117]

11 **Käpyaho K**, Welin MG, Tanner P, Kärkkäinen T, Weber T. Rapid determination of C-reactive protein by enzyme immunoassay using two monoclonal antibodies. *Scand J Clin Lab Invest* 1989; **49**: 389-393 [PMID: 2662385 DOI: 10.3109/00365518909089112]

12 **Chandra P**, Noh HB, Shim YB. Cancer cell detection based on the interaction between an anticancer drug and cell membrane components. *Chem Commun (Camb)* 2013; **49**: 1900-1902 [PMID: 23296144 DOI: 10.1039/c2cc38235kPMID: 23296144]

13 **Chandra P**, Noh HB, Won MS, Shim YB. Detection of daunomycin using phosphatidylserine and aptamer co-immobilized on Au nanoparticles deposited conducting polymer. *Biosens Bioelectron* 2011; **26**: 4442-4449 [PMID: 21612907 DOI: 10.1016/j.bios.2011.04.060]

14 **Noh HB**, Chandra P, Moon JO, Shim YB. In vivo detection of glutathione disulfide and oxidative stress monitoring using a biosensor. *Biomaterials* 2012; **33**: 2600-2607 [PMID: 22209642 DOI: 10.1016/j.biomaterials.2011.12.026]

15 **Kim HC**, Lee SK, Jeon WB, Lyu HK, Lee SW, Jeong SW. Detection of C-reactive protein on a functional poly(thiophene) self-assembled monolayer using surface plasmon resonance. *Ultramicroscopy* 2008; **108**: 1379-1383 [PMID: 18565665 DOI: 10.1016/j.ultramic.2008.04.052]

16 **Lee MH**, Lee KN, Jung SW, Kim WH, Shin KS, Seong WK. Quantitative measurements of C-reactive protein using silicon nanowire arrays. *Int J Nanomedicine* 2008; **3**: 117-124 [PMID: 18488422]

17 **Yuan H**, Kwon HC, Yeom SH, Kwon DH, Kang SW. MOSFET-BJT hybrid mode of the gated lateral bipolar junction transistor for C-reactive protein detection. *Biosens Bioelectron* 2011; **28**: 434-437 [PMID: 21835604 DOI: 10.1016/j.bios.2011.07.062PMID: 21835604]

18 **Zhou F**, Lu M, Wang W, Bian ZP, Zhang JR, Zhu JJ. Electrochemical immunosensor for simultaneous detection of dual cardiac markers based on a poly(dimethylsiloxane)-gold nanoparticles composite microfluidic chip: a proof of principle. *Clin Chem* 2010; **56**: 1701-1707 [PMID: 20852134 DOI: 10.1373/clinchem.2010.147256PMID: 20852134]

19 **Cowles CL**, Zhu X. Sensitive detection of cardiac biomarker using ZnS nanoparticles as novel signal transducers. *Biosens Bioelectron* 2011; **30**: 342-346 [PMID: 22014622 DOI: 10.1016/j.bios.2011.09.034]

20 **Qureshi A**, Roci I, Gurbuz Y, Niazi JH. An aptamer based competition assay for protein detection using CNT activated gold-interdigitated capacitor arrays. *Biosens Bioelectron* 2012; **34**: 165-170 [PMID: 22365749 DOI: 10.1016/j.bios.2012.01.038]

21 **Kim CH**, Ahn JH, Kim JY, Choi JM, Lim KC, Park TJ, Heo NS, Lee HG, Kim JW, Choi YK. CRP detection from serum for chip-based point-of-care testing system. *Biosens Bioelectron* 2013; **41**: 322-327 [PMID: 23017687 DOI: 10.1016/j.bios.2012.08.047PMID: 23017687]

22 **Oh YK**, Joung HA, Kim S, Kim MG. Vertical flow immunoassay (VFA) biosensor for a rapid one-step immunoassay. *Lab Chip* 2013; **13**: 768-772 [PMID: 23303290 DOI: 10.1039/c2lc41016h]

23 **Bryan T**, Luo X, Bueno PR, Davis JJ. An optimised electrochemical biosensor for the label-free detection of C-reactive protein in blood. *Biosens Bioelectron* 2013; **39**: 94-98 [PMID: 22809521 DOI: 10.1016/j.bios.2012.06.051]

24 **Chen CS**, Chang KN, Chen YH, Lee CK, Lee BY, Lee AS. Development of a label-free impedance biosensor for detection of antibody-antigen interactions based on a novel conductive linker. *Biosens Bioelectron* 2011; **26**: 3072-3076 [PMID: 21185166 DOI: 10.1016/j.bios]

**P-Reviewers:** **Ayroldi E,** Miller GP, Ria R, Shafer LA **S-Editor:** Qi Y

**L-Editor: E-Editor:**

Figure 1 Diagrammatic representation of the advancement in the C-reactive protein detection.

Table 1 Various C-reactive protein detection techniques and their characteristics

|  |  |  |  |
| --- | --- | --- | --- |
| **No.** | **Technique employed** | **Features** | **Ref.** |
| 1. | Radial Immunodiffusion | Qualitative analysis in less than 48 h. | Harris *et al*[8]1984 |
| 2. | Latex Agglutination | Time taken less than 24 h; qualitative analysis. | Senju *et al*[9]*,* 1986 |
| 3. | Latex piezoelectric Assay | Uses quartz crystal and latex bearing antibody; sensitive than conventional methods; less time required. | Kurosawa *et al*[10]*,* 1990 |
| 4. | Immuno-enzymometric Immunoassay | Single immunological reaction; sensitive; results comparable to turbidimetric detection. | Kapyaho *et al*[11]*.,* 1990 |
| 5. | Surface plasma resonance spectrophotometry | High sensitivity; on-site analysis; SAM usage. | Kim *et al*[15] 2008 |
| 6. | Silicon nanowire based assays | Micro-machining technology; higher detection limit. | Min-Ho *et al*[16] 2008 |
| 7. | MOFSET/BJT based technique | High sensitivity, change in capacitance measurement; reliable; small size; ease of manufacturing; god selectivity; highly reproducible; high transconductivity. | Yuan *et al*[17] 2011 |
| 8. | Electrochemical Immunosensor | Detection by square wave stripping voltammetry; quantitative analysis of 2 biomarkers; reproducible. | Zhou *et al*[18]*,* 2010 |
| 9. | Nanotechnology using ZnS nanoparticles | Detection by fluorescence spectrophotometry; highly sensitive; non-toxic; low cast system; highly specific. | Chad *et al*[19]*,* 2011 |
| 10. | RNA Aptamer based technology | Uses Carbon nanotube’s interdigitated electrodes of capacitors; highly selective.  | Quereshi *et al*[20]*,* 2012 |
| 11. | Biosensor using FET (Field emission transmitter) | Involves SBP linked in protein A; point of care testing system; on-site analysis. | Kim *et al*[21]*,* 2013 |
| 12. | Vertical flow Immunoassay | One-step assay; time taken 2 minutes; most rapid; employs gold nanoparticles. | Oh *et al*[22] 2013 |
| 13. | Electrochemical impedance Spectroscopy | Most advanced technique; uses gold and diamond spray in fabrication; highly sensitive; reusable without sensitivity being lost; good detection limit. | Bryan *et al*[23]*,* 2013 |